Parent

Online European Patent Register - Results

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Choose your View: All data mentioned in Rule 92 and EPIDOS

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Most recent event
Application deemed to be withdrawn
Date of publication 30-06-2004 [2004/27]

Publication numbers, publication type and publication dates
EP0822255 A2 04-02-1998 [1998/06]
EP0822255 A3 27-10-1999 [1999/43]

Application numbers and filing date
EP19970201842 (97201842.8)
Date of filing 17-10-1991 [1998/06]

Date of publication of search report
Date of publication of search report 27-10-1999 [1999/43]

Priority number, priority date
GB19900022543 17-10-1990 [1998/06]

Classification (IPC) and bulletin number
C12N15/13, C07K16/28, A61K39/395, C12N5/10 [1998/06]

Designated states
AT , BE , CH , DE , DK , ES , FR , GB , GR , IT , LI , LU , NL , SE [1998/06]

English title
CHO (Chinese Hamster Ovary) glycosylated antibodies and their use in therapy [1998/06]

French title
Anticorps glycosylés produits à partir de cellules CHO (Chinese Hamster Ovary) et leurs utilisations thérapeutiques [1998/06]

German title
Von CHO (Chinese Hamster Ovary) glykosylierte Antikörper und ihre therapeutische Verwendung [1998/06]

Designated states, applicant name, address
FOR ALL DESIGNATED STATES
THE WELLCOME FOUNDATION LIMITED
Glaxo Wellcome House, Berkeley Avenue
Greenford, Middlesex UB6 0NN/GB [1998/06]

Inventor name, address
01 / Page, Martin John / Glaxo Res. and Dev. Ltd., Gunnels Wood Road / Stevenage, Hertfordshire SG1 2NY / GB [1998/06]

Representative name, address
Stott, Michael John, et al
GlaxoSmithKline Corporate Intellectual Property (CN9.25.1) 980 Great West Road
Brentford, Middlesex TW8 9GS/GB [1998/06]

Filing language
EN

Procedure language
EN

Publication language
A2 EN [1998/06]

Location of file and fax number for file inspection requests
Application is treated in (/fax-nr) MUNICH/(+49-89) 23994465

Examination procedure
request for examination 24-06-1997 [1998/06]
Examination report(s) A.96(2), R.51(2) date dispatch/time-limit/reply 05-08-2003/M04/00000000

Application withdrawn or deemed to be withdrawn
Communication, that the application is deemed to be withdrawn date dispatch/legal effect

date	28-01-2004/16-12-2003 [2004/27]
Reason	A.96(3)
Request for further processing - date of filing / fee payment	A.121 02-10-2000 / 02-10-2000
- decision / date	request accepted / 17-10-2000

Earlier Application

Parent application/publication number(s)	EP19910309595/EP0481790 [1998/06]

Divisional Application(s)

Divisional application/publication number(s)	EP20020076251/EP1247865 EP20040077314/EP1484402

Renewal fees

Renewal fee A.86 (patent year / paid)	03/24-06-1997 04/24-06-1997 05/24-06-1997 06/24-06-1997 07/10-10-1997 08/12-10-1998 09/14-10-1999 10/11-10-2000 11/11-10-2001 12/15-10-2002 13/06-10-2003

Documents cited in the European Search

EP0388151 A1 [X];
EP0325262 A2 [Y];
EP0365209 A2 [A];
XP002112008 A [Y];
XP002112009 A [Y];
XP002112010 A [XP]

[Y] KAETZEL, D.M. ET AL.: "Methotrexate-induced amplification of the bovine lutropin genes in chinese hamster ovary cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 263, no. 13, 5 May 1988 (1988-05-05), pages 6344-6355, XP002112008 MD US

[Y] KAUFMAN, R.: "Gene expression technology; Selection and coamplification of heterologous genes in mammalian cells" METHODS IN ENZYMOLOGY, vol. 185, 1989, pages 537-566, XP002112009

[XP] WOOD, CLIVE R. ET AL: "High level synthesis of immunoglobulins in chinese hamster ovary cells" JOURNAL OF IMMUNOLOGY, vol. 145, no. 9, November 1990 (1990-11), pages 3011-3016, XP002112010 BALTIMORE US

[End of Data]

Return to Search Screen
15-02-2006 09:58:15

Antrag auf Erteilung eines europäischen Patents / Request for grant of a European patent / Requête en délivrance d'un brevet européen

1

Bestätigung einer bereits durch Telekopie (Telefax) eingereichten Anmeldung / Confirmation of an application already filed by facsimile / Confirmation d'une demande déjà déposée par télécopie
Wenn ja, Datum der Übermittlung der Telekopie und Name der Einreichungsbehörde / If yes, facsimile date and name of the authority with which the documents were filed / Si oui, date d'envoi de la télécopie et nom de l'autorité de dépôt

☐ Ja / Yes / Oui

Datum / Date | Behörde / Authority / Autorité

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Anmeldenummer / Application No / N° de la demande	MKEY	1	97201842.8
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	2	24/6/97
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA	3	
Anmeldetag / Date of filing / Date de dépôt		4	

Tabulatoren-Positionen / Tabulation marks / Arrêts de tabulation

Es wird die Erteilung eines europäischen Patents und gemäß Artikel 94 die Prüfung der Anmeldung beantragt / Grant of a European patent, and examination of the application under Article 94, are hereby requested / Il est demandé la délivrance d'un brevet européen et, conformément à l'article 94, l'examen de la demande

EXAM 4 | 5 ☒ *Prüfungsantrag in einer zugelassenen Nichtamtssprache (siehe Merkblatt II, 5) / Request for examination in an admissible non-EPO language (see Notes II,5) / Requête en examen dans une langue non officielle autorisée (voir notice II,5)*

Zeichen des Anmelders oder Vertreters (max 15 Positionen) / **Applicant's or representative's reference** (maximum 15 spaces) / **Référence du demandeur ou du mandataire** (max 15 caractères ou espaces)

AREF | 6 PA1195/EP2

ANMELDER / APPLICANT / DEMANDEUR
Name / Nom 7 The Wellcome Foundation Limited

Anschrift / Address / Adresse 8
Glaxo Wellcome House
Berkeley Avenue
Greenford
Middlesex
UB6 0NN
United Kingdom

APPR 01 # 20151766
DEST

Zustellanschrift / Address for correspondence / Adresse pour la correspondance 9
Glaxo Wellcome House
Berkeley Avenue
Greenford
Middlesex
UB6 0NN
United Kingdom

PADR

Staat des Wohnsitzes oder Sitzes / State of residence or of principal place of business / Etat du domicile ou du siège 10 GB

Staatsangehörigkeit / Nationality / Nationalité 11 GB

Telefon / Telephone / Téléphone 12 0171 493 4060

Telex / Télex | **Telefax / Fax / Téléfax** 13 25456 | 0181 966 8838

Weitere(r) Anmelder auf Zusatzblatt / Additional applicant(s) on additional sheet / Autre(s) demandeur(s) sur feuille additionnelle 14 ☐

VERTRETER / REPRESENTATIVE / MANDATAIRE:
Name / Nom 15 Michael J STOTT

(Nur einen Vertreter angeben, der in das europäische Patentregister eingetragen und an den zugestellt wird / Name **only one** representative, who is to be listed in the Register of European Patents and to whom notification is to be made / N'indiquer qu'un **seul** mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)

FREP 01 | 04137732 # | # 1 #

Geschäftsanschrift / Address of place of business / Adresse professionnelle 16
Glaxo Wellcome plc
Glaxo Wellcome House
Berkeley Avenue
Greenford
Middlesex
UB6 0NN
United Kingdom

Telefon / Telephone / Téléphone 17 0171 493 4060

Telex / Télex | **Telefax / Fax / Téléfax** 18 25456 | 0181 966 8838

Weitere(r) Vertreter auf Zusatzblatt / Additional representative(s) on additional sheet / Autre(s) mandataire(s) sur feuille additionnelle 19 x

TRAN | FILL EN

REQUEST

Vollmacht / Authorisation / Pouvoir: **ist beigefügt / is enclosed / ci-joint**		20	☐
ist registriert unter Nummer / has been registered under No. / a été enregistré sous le n°	GENA	21	Nummer / Number / Numéro
ERFINDER / INVENTOR / INVENTEUR:	INVT 20 # #		
Anmelder ist (sind) alleinige(r) Erfinder / The applicant(s) is (are) the sole inventor(s) / Le(s) demandeur(s) est (sont) le (les) seul(s) inventeur(s)		22	☐
Erfindernennung auf gesondertem Schriftstück / Designation of inventor attached / Voir la désignation de l'inventeur ci-jointe		23	x
BEZEICHNUNG DER ERFINDUNG / TITLE OF INVENTION / TITRE DE L'INVENTION:	TIDE TIEN TIFR	24	Antibody for use in therapy

PRIORITÄTSERKLÄRUNG / DECLARATION OF PRIORITY / DECLARATION DE PRIORITE	PRIO	25	Staat / State / Etat	Anmeldetag / Filing date / Date de dépôt	Aktenzeichen / Application No / N° de la demande
01 # # #			1 GB	17.10.00	4
01/GB/17.10.90(17.10.91)/ GB 9022543(17.10.91)/EN(17.10.91)/00.00.00					
03 # # . #			3		
04 # . # # . .			4		
Weitere Prioritatserklärung(en) auf Zusatzblatt / Additional declaration(s) of priority on additional sheet / Autre(s) déclaration(s) de priorité sur feuille additionnelle			☐		

BIOLOGISCHES MATERIAL Die Erfindung betrifft biologisches Material oder seine Verwendung, das nach Regel 28 hinterlegt worden ist	**BIOLOGICAL MATERIAL** The invention relates to and/or uses biological material deposited under Rule 28	26	☐ **MATIERE BIOLOGIQUE** L'invention concerne et/ou utilise la matière biologique, déposée conformément à la règle 28
BIOM 1 # #			

Die Angaben nach Regel 28(1) c) sind in den technischen Anmeldungsunterlagen enthalten auf / The particulars referred to in Rule 28(1) (c) are given in the technical documents in the application on / Les indications visées à la règle 28(1) c) figurent dans les pièces techniques de la demande à la /aux		27	Seite(n) / page(s) / Zeile(n) / line(s) / ligne(s)
werden später mitgeteilt / will be submitted later / seront communiquées ultérieurement		27a	☐
Die Empfangsbescheinigung(en) der Hinterlegungsstelle ist (sind) beigefügt / The receipt(s) of deposit issued by the depositary institution is (are) enclosed / Le(s) récépissé(s) de dépôt délivré(s) par l'autorité de dépôt est (sont) ci-joint(s)		28	☐
wird (werden) nachgereicht / will be filed later / sera (seront) produit(s) ultérieurement		28a	☐
Verzicht auf die Verpflichtung des Antragstellers nach Regel 28(3) auf gesondertem Schriftstück / Waiver of the right to an undertaking from the requester pursuant to Rule 28(3) attached / Renonciation, sur document distinct, à l'engagement du requérant au titre de la règle 28(3)		29	☐

Falls das biologische Material nicht vom Anmelder, sondern von einem Dritten hinterlegt wurde: / Where the biological material has been deposited by a person other than the applicant: / Lorsque la matière biologique a été déposée par une personne autre que le demandeur: 30

Name und Anschrift des Hinterlegers / Name and address of depositor / Nom et adresse du déposant

Ermächtigung nach Regel 28(1)d) / Authorisation under Rule 28(1)(d) / Autorisation en vertu de la règle 28(1)d)

ist beigefügt / is enclosed / ci-jointe 30a ☐

wird nachgereicht / will be filed later / sera produite ultérieurement 30b ☐

NUCLEOTID-UND AMINOSÄURESEQUENZEN / NUCLEOTIDE AND AMINO ACID SEQUENCES / SEQUENCES DE NUCLEOTIDES ET D'ACIDES AMINES SEQL (1) 31

Die Beschreibung enthält ein Sequenzprotokoll nach Regel 27a(1) / The description contains a sequence listing in accordance with Rule 27a(1) / La description contient une liste de séquences selon la règle 27bis(1) ☐

Der vorgeschriebene maschinenlesbare Datenträger ist beigefügt / The prescribed machine readable data carrier is enclosed / Le support de données prescrit déchiffrable par machine est annexé ☐

Es wird hiermit erklärt, daß die auf dem Datenträger gespeicherte Information mit dem schriftlichen Sequenzprotokoll übereinstimmt (Regel 27a(2)) / It is hereby stated that the information recorded on the data carrier is identical to the written sequence listing (Rule 27a(2)) /Il est déclaré par la présente que l'information figurant sur le support de données est identique à celle que contient la liste de séquences écrite (règle 27bis (2)) ☐

Verschiedene Anmelder für verschiedene Vertragsstaaten / Different applicants for different Contracting States / Différents demandeurs pour différents Etats contractants 32

Name(n) des (der) Anmelder(s) und benannte Vertragsstaaten / Name(s) of applicant(s) and designated Contracting States / Nom(s) du (des) demandeur(s) et des Etats contractants désignés

APPR 02 # | | | | | | | | # | | | | | | | | | | | | | |

BENENNUNG VON VERTRAGSSTAATEN / DESIGNATION OF CONTRACTING STATES / DESIGNATION D'ETATS CONTRACTANTS DEST 33

Österreich / Austria / Autriche	AT	x
Belgien / Belgium / Belgique	BE	x
Schweiz und Liechtenstein / Switzerland and Liechtenstein / Suisse et Liechtenstein	CH / LI	x
Deutschland / Germany / Allemagne	DE	x
Dänemark / Denmark / Danemark	DK	x
Spanien / Spain / Espagne	ES	x
Finnland / Finland / Finlande	FI	
Frankreich / France / France	FR	x
Vereinigtes Königreich / United Kingdom / Royaume-Uni	GB	x
Griechenland / Greece / Grèce	GR	x
Irland / Ireland / Irlande	IE	
Italien / Italy / Italie	IT	x
Luxemburg / Luxembourg / Luxembourg	LU	x
Monaco / Monaco / Monaco	MC	
Niederlande / Netherlands / Pays-Bas	NL	x
Portugal / Portugal / Portugal	PT	
Schweden / Sweden / Suède	SE	x
_____		
_____		

(Platz für Vertragsstaaten, für die das EPÜ nach Drucklegung dieses Formblatts in Kraft tritt) (Space for Contracting States for which the EPC enters into force after this form has been printed) (Prévu pour des Etats contractants à l'égard desquels la CBE entrera en vigueur après l'impression du présent formulaire)

PA1195/EP2

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

VORSORGLICHE BENENNUNG SÄMTLICHER VERTRAGSSTAATEN
Die in Feld 33 angegebenen Staaten sind jene, für die die Zahlung der Benennungsgebühren vorgenommen wurde oder derzeit beabsichtigt ist Vorsorglich werden jedoch sämtliche Staaten benannt, die zum Zeitpunkt der Einreichung dieser Anmeldung Vertragsstaaten des EPÜ sind (Derzeit AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE) Es wird ersucht, die Benennung der hier zusätzlich benannten Vertragsstaaten als vom Anmelder zurückgenommen zu betrachten, wenn für diese Staaten die Benennungsgebühren nicht bis zum Ablauf der in Regel 85a(2) vorgesehenen Nachfrist entrichtet werden Es wird beantragt, von der Zustellung einer Mitteilung nach Regel 85a(1) und einer Mitteilung nach Regel 69(1) betreffend die hier zusätzlich benannten Vertragsstaaten abzusehen

PRECAUTIONARY DESIGNATION OF ALL CONTRACTING STATES
The States indicated in Section 33 are those for which it is at present intended to pay designation fees if these have not already been paid As a precautionary measure, however, all those States which are Contracting States to the EPC at the time of filing this application are designated (Present situation AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE) It is hereby requested that the designation of any additional States thereby included be regarded as withdrawn by the applicant if the designation fees have not been paid by the time the period of grace allowed in Rule 85a(2) expires It is requested that no communication under Rule 85a(1) nor any communication under Rule 69(1) concerning the additional Contracting States designated above be notified

33a ☒

DÉSIGNATION A TOUTES FINS UTILES DE TOUS LES ETATS CONTRACTANTS
Les Etats indiqués à la rubrique 33 sont ceux pour lesquels le paiement des taxes de désignation a été effectué ou pour lesquels l'on se propose actuellement de payer les taxes de désignation Toutefois, à toutes fins utiles, sont désignés tous les Etats qui sont des Etats contractants de la CBE à la date du dépôt de la demande (Situation actuelle AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE) Il est demandé, au cas où les taxes de désignation pour les Etats contractants désignés à titre complémentaire ne seraient pas acquittées dans le délai supplémentaire prévu à la règle 85bis(2), que la désignation desdits Etats soit considérée comme retirée par le demandeur Prière de ne pas procéder pour lesdits Etats contractants désignés à titre complémentaire à la signification d'une notification établie conformément à la règle 85bis(1) ou à la règle 69(1)

ERSTRECKUNG DES EUROPÄISCHEN PATENTS

Diese Anmeldung gilt als Antrag, die europäische Patentanmeldung und das darauf erteilte europäische Patent auf alle Nicht-Vertragsstaaten des EPÜ zu erstrecken, mit denen am Tag ihrer Einreichung „Erstreckungsabkommen" bestehen
(Derzeit Albanien, Litauen, Lettland, Slowenien)
Die Erstreckung wird jedoch nur wirksam, wenn die vorgeschriebene Erstreckungsgebühr entrichtet wird

EXTENSION OF THE EUROPEAN PATENT

This application is deemed to be a request to extend the European patent application and the European patent granted in respect of it to all non-Contracting States to the EPC with which "extension agreements" exist on the date on which the application is filed (Present situation Albania, Lithuania, Latvia, Slovenia) However, the extension only takes effect if the prescribed extension fee is paid

EXPT

34

EXTENSION DES EFFETS DU BREVET EUROPEEN

La présente demande est réputée constituer une requête en extension des effets de la demande de brevet européen et du brevet européen délivré sur la base de cette demande à tous les Etats non parties à la CBE avec lesquels il existe un «accord d'extension» à la date du dépôt de la demande (Situation actuelle Albanie, Lituanie, Lettonie, Slovénie) Toutefois l'extension ne produit ses effets que s'il est acquitté la taxe d'extension prescrite

Der Anmelder beabsichtigt derzeit, die Erstreckungsgebühr für die nachfolgend angekreuzten Staaten zu entrichten: / The applicant currently intends to pay the extension fee for the States marked below with a cross: / Le demandeur se propose actuellement d'acquitter la taxe d'extension pour les Etats dont le nom est coché ci-après :

Albanien / Albania / Albanie	AL	
Litauen / Lithuania / Lituanie	LT	
Lettland / Latvia / Lettonie	LV	
Slowenien / Slovenia / Slovénie	SI	

(Platz für Staaten, mit denen nach Drucklegung dieses Formblatts „Erstreckungsabkommen" in Kraft treten) / (Space for States with which "extension agreements" enter into force after this form has been printed) / (Prévu pour des Etats à l'égard desquels des «accords d'extension» entreront en vigueur après l'impression du présent formulaire)

Die Anmeldung ist eine Teilanmeldung / The application is a divisional application / La présente demande constitue une demande divisionnaire

DFIL 9 17 10 91 #
PANR 91309595 6 #

35 x 91309595.6 Nummer der früheren Anmeldung / No of earlier application / Numéro de la demande initiale

Es handelt sich um eine Anmeldung nach Art. 61(1)b) / The application is an Art. 61(1)(b) application / La présente demande constitue une demande selon l'article 61(1)b)

DFIL 9 #
EANR #

36 Nummer der früheren Anmeldung / No of earlier application / Numéro de la demande initiale

Patentansprüche / Claims / Revendications CLMS

37 Zahl der Patentansprüche / Number of claims / Nombre de revendications

Zur Veröffentlichung mit der Zusammenfassung wird vorgeschlagen Abbildung Nr. / With the abstract it is proposed to publish figure No. / Il est proposé de publier avec l'abrégé la figure n° DRAW (2)

39 Nummer / Number / Numéro

Zusätzliche Abschrift(en) der im europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt / Additional copy(ies) of the documents cited in the European search report is (are) requested / Prière de fournir une (des) copie(s) supplémentaire(s) des documents cités dans le rapport de recherche européenne

ASOC

40 [ ]

Anzahl der **zusätzlichen** Sätze von Abschriften
Number of **additional** sets of copies
Nombre de jeux **supplémentaires** de copies

Es wird die Rückerstattung der Recherchengebühr gemäß Art. 10 GebO beantragt / Refund of the search fee is requested pursuant to Article 10 of the Rules relating to Fees / Le remboursement de la taxe de recherche est demandé en vertu de l'article 10 du règlement relatif aux taxes

41 [ ]

Eine Kopie des Recherchenberichts ist beigefügt / A copy of the search report is attached / Une copie du rapport de recherche est jointe

42 [ ]

AUTOMATISCHER ABBUCHUNGSAUFTRAG *(nur möglich für Inhaber von beim EPA geführten laufenden Konten)*
AUTOMATIC DEBIT ORDER *(for EPO deposit account holders only)*
ORDRE DE PRELEVEMENT AUTOMATIQUE *(uniquement possible pour les titulaires de comptes courants ouverts auprès de l'OEB)*

Das Europäische Patentamt wird hiermit beauftragt, fällig werdende Gebühren und Auslagen nach Maßgabe der Vorschriften über das automatische Abbuchungsverfahren vom nebenstehenden laufenden Konto abzubuchen / The European Patent Office is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account opposite any fees and costs falling due / Par la présente, il est demandé à l'Office européen des brevets de prélever du compte courant ci-contre les taxes et frais venant à échéance, conformément à la réglementation relative au prélèvement automatique

DECA

FÜR AUTOMATISCHEN ABBUCHUNGSAUFTRAG:
FOR AUTOMATIC DEBIT ORDER:
POUR L'ORDRE DE PRELEVEMENT AUTOMATIQUE:

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant

43 [ ]

Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte

Eventuelle RÜCKZAHLUNGEN auf das nebenstehende beim EPA geführte laufende Konto / REIMBURSEMENT, if any, to EPO deposit account opposite / REMBOURSEMENTS éventuels à effectuer sur le compte courant ci-contre ouvert auprès de l'OEB

DEPA

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant

44 28050185

Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte

Glaxo Wellcome plc

Die vorgeschriebene Liste über die diesem Antrag beigefügten Unterlagen ergibt sich aus der vorbereiteten Empfangsbescheinigung (Seite 6 dieses Antrages)

The prescribed list of documents enclosed with this request is shown on the prepared receipt (page 6 of this request)

45

La liste prescrite des documents joints à cette requête figure sur le récépissé préétabli (page 6 de la présente requête)

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) / Signature(s) of applicant(s) or representative(s) / Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

46

Für Angestellte nach Artikel 133(3) Satz 1 mit allgemeiner Vollmacht / For employees under Article 133(3), 1st sentence, having a general authorisation / Pour les employés mentionnés à l'article 133(3), 1ère phrase, munis d'un pouvoir général
Nr. / No. / n° :

Ort / Place / Lieu Greenford, UK

Datum / Date 19 June 1997

Michael J STOTT
Representative

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben / Please type name under signature. In case of legal persons, the position of the signatory within the company should also be typed / Le ou les noms des signataires doivent être également dactylographiés. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire

PA1195/EP2

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Empfangsbescheinigung / Receipt for documents / Récépissé de documents 6

(Liste der diesem Antrag beigefügten Unterlagen / Checklist of enclosed documents / Liste des documents annexés à la présente requête)

Es wird hiermit der Empfang der unten bezeichneten Dokumente bescheinigt / Receipt of the documents indicated below is hereby acknowledged / Nous attestons le dépôt des documents désignés ci-dessous

Wird im Falle der Einreichung der europäischen Patentanmeldung bei einer nationalen Behörde diese Empfangsbescheinigung vom Europäischen Patentamt übersandt, so ist sie als Mitteilung gemäß Regel 24(4) anzusehen (siehe Feld RENA) **Nach Erhalt der Mitteilung nach Regel 24(4) sind alle weiteren Unterlagen, die die Anmeldung betreffen, nur noch unmittelbar beim EPA einzureichen.** / If this receipt is issued by the European Patent Office and the European patent application was filed with a national authority it serves as a communication under Rule 24(4) (see Section RENA) **Once the communication under Rule 24(4) has been received, all further documents relating to the application must be sent directly to the European Patent Office.** / Si, en cas de dépôt de la demande de brevet européen auprès d'un service national, l'Office européen des brevets délivre le présent récépissé de documents, ce récépissé est réputé être la notification visée à la règle 24(4) **Dès que la notification visée à la règle 24(4) a été reçue, tous les autres documents relatifs à la demande doivent être adressés directement à l'OEB.**

Michael J STOTT
Glaxo Wellcome plc
Glaxo Wellcome House
Berkeley Avenue
Greenford
Middlesex
UB6 0NN
United Kingdom

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Datum / Date 30.06.97

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Anmeldenummer / Application No. / N° de la demande			97201842.8
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC		24.06.97
Zeichen des Anmelders/Vertreters / Applicant's/ Representative's ref / Référence du demandeur ou du mandataire	AREF		
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A.	**Anmeldungsunterlagen und Prioritätsbeleg(e) / Application documents and priority document(s) / Pièces de la demande et document(s) de priorité**				
1	Beschreibung / Description		3	28	
2	Patentanspruche / Claim(s) / Revendication(s)		3	2	
3	Zeichnung(en) / Drawing(s) / Dessin(s) DRAW 1 #				
4	Zusammenfassung / Abstract / Abrégé				
5	Übersetzung der Anmeldungsunterlagen / Translation of the application documents / Traduction des pièces de la demande				
6	Prioritatsbeleg(e) / Priority document(s) / Document(s) de priorité				
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5	Gebührenzahlungsvordruck (EPA Form 1010) / Voucher for the settlement of fees (EPO Form 1010) / Bordereau de règlement de taxes (OEB Form 1010)		X	
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Wahrung Betrag / Currency Amount / Monnaie Montant
(Ausfullung freigestellt / optional / facultatif)
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PA1195/EP2

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Dear Sirs,

New European Patent Application
Divisional of European Application No. 91309595.6
Applicant: The Wellcome Foundation Limited

Please find enclosed the following documents in respect of the above-mentioned divisional patent application:

- Form 1001
- Form 1002
- Description and claims (30 pages) in triplicate
- Form 1010 showing fee payable DM14130. The Receiving Office is hereby authorised to charge any deficiency in the total fees indicated to deposit account no. 28050185
- Copy of search report relating to European Patent Application No. 91309595.6
- Form 1037.

Yours faithfully,

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EP 0 481 790 A3

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 91 30 9595

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
Y	WO-A-8 901 975 (CELLTECH, LDT.) 9 March 1989 * the whole document * ---	1-23	C12N15/13 C12P21/08 C12N5/10 A61K39/395 //C12N5/10 C12R1:91) (C12P21/08 C12R1:91)
Y	JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS) vol. 263, no. 13, 5 May 1988, BALTIMORE, MD US pages 6344 - 6355 Kaetzel, D.M.; Nilson, J.H.; 'Methotrexate-induced amplification of the bovine lutropin gens in chines hamster ovary cells.' ---	1-23	
Y	METHODS IN ENZYMOLOGY vol. 185, 1989, pages 537 - 566 KAUFMAN, R.; 'Gene Expression technology; Selection and coamplification of heterologous genes in mammalian cells' --- -/--	1-23	

TECHNICAL FIELDS SEARCHED (Int. Cl. 5)

C12N
C07K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely:

Claims searched incompletely:

Claims not searched:

Reason for the limitation of the search:

see sheet C

KOPIE IN RECHERCHEAKTE

Place of search	Date of completion of the search	Examiner
THE HAGUE	08 FEBRUARY 1993	NAUCHE S.A.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0427)

EP 0 481 790 A3

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

EP 91 30 9595

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
P,X	JOURNAL OF IMMUNOLOGY. vol. 145, no. 9, 1 November 1990, BALTIMORE US pages 3011 - 3016 Wood, Clive R.; Dorner, Andrew J.; Morris, George E.; Alderman, Edward M.; Wilson, Douglas; O'Hara, Richard M., Jr.; Kaufman, Rand 'High level synthesis of immunoglobulins in Chinese hamster ovary cells.' * the whole document * ---	1-23	
A	EP-A-0 365 209 (BECTON DICKINSON AND COMPANY, US) 25 April 1990 -----		TECHNICAL FIELDS SEARCHED (Int. Cl. 5)

EPO FORM 1503 03.82 (P0401)

Sheet C EP 91309595

Remark:
Although claims 19 and 21 partially are directed to a method of treatment of the human body (Article 52(4) EPC) the search has been carried out and based on the alleged effects of the compound/composition.

ANTIBODY FOR USE IN THERAPY

The present invention relates to antibodies having CHO glycosylation for use in human medical therapy, and formulations containing such antibodies.

Antibodies or immunoglobulins are proteinaceous bifunctional molecules. One region which is highly variable between different antibodies is responsible for binding to an antigen (Fab region), for example the many different infectious agents that the body may encounter, whilst the second, constant region (or Fc region) is responsible for binding to the Fc receptors of cells and also activates complement. In this way, antibodies represent a vital component of the immune response of mammals in destroying foreign microorganisms and viruses.

An antibody molecule is composed of two light chains and two heavy chains that are held together by interchain disulphide bonds. Each light chain is linked to a heavy chain by disulphide bonds and the two heavy chains are linked to each other by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains, and each light chain has a variable domain at one end and a constant domain at the other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The remaining constant domains of the heavy chains are aligned with each other. The constant domains in the light and heavy chains are not involved directly in binding the antibody to the antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. They have the same general structure with each domain comprising a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet

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conformation and the CDRs form loops connecting, and in some cases comprising part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site.

The immunisation of an animal with an antigen results in the production of different antibodies with different specificities and affinities. An antiserum obtained from the immunised animal will, therefore, be heterogeneous and contain a pool of antibodies produced by many different lymphocyte clones. Antibodies thus obtained are referred to as polyclonal antibodies and this polyclonal nature has been a major drawback in the use of antibodies in diagnostic assays and in therapeutic applications.

A major step forward occurred in 1975 when Kohler and Milstein (Nature, 1975, 256, 495-497) reported the successful fusion of spleen cells from mice immunized with an antigen with cells of a murine myeloma line. The resulting hybrid cells, termed hybridomas, have the properties of antibody production derived from spleen cells and of continous growth derived from the myeloma cells. Each hybridoma synthesizes and secretes a single antibody to a particular determinant of the original antigen. To ensure that all cells in a culture are identical, i.e. that they contain the genetic information required for the synthesis of a unique antibody species, the hybridomas resulting from cell fusion are cloned and subcloned. In this way, the cloned hybridomas produce homogeneous or monoclonal antibodies.

The advantages of hybridoma technology are profound. Because many hybrids arising from each spleen are screened for their potential to produce antibodies to the antigen of interest and only a few are selected, it is possible to immunize with impure antigens and yet obtain specific antibodies. The immortality of the cell line assures that an unlimited supply of a homogeneous, well-characterised antibody is available for use in a variety of applications including in particular diagnosis and immunotherapy of pathological disorders.

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Unfortunately, the usefulness of such monoclonal antibodies in a clinical setting can be severely hampered by the development of human anti-mouse antibodies - an anti-globulin response - which may interfere with therapy or cause allergic or immune complex hypersensitivity.

When, for example, murine (or ratine) monoclonal antibodies are used in human therapy, the induction of an human anti-mouse antibody response is due to the murine origin of the constant domains and four framework regions. This problem has therefore been addressed by the development of antibodies of two basic types. The first type, referred to as chimeric antibodies, is where the murine constant domains only are replaced by equivalent domains of human origin (Morrison et al, P.N.A.S., 1984, 81, 6851-6855; Boulianne et al, Nature, 1985, 314, 268-270; and Neuberger et al, Nature, 1985, 314, 268-270). The second type is where the murine constant domains and the murine framework regions are all replaced by equivalent domains and regions of human origin. This second type of antibody is referred to as a humanised or CDR-grafted antibody (Jones et al, Nature, 1986, 321, 522-525; and Riechmann et al, Nature, 1988, 332, 323-327). A human antibody would of course avoid the need for "humanisation", however cell lines which secrete human antibodies are very unstable and have generally proven unsuitable for commercial scale production.

To generate sufficient quantities of antibody for full clinical use it is desirable to employ an efficient recombinant expression system. Since myeloma cells represent a natural host specialized for antibody production and secretion, cell lines derived from these have been used for the expression of recombinant antibodies. Often, complex vector design, based around immunoglobulin gene regulatory elements, is required, and final expression levels have been reported which are highly variable (Winter et al, Nature, 1988, 332, 323-327; Weidle et al, Gene, 1987, 60, 205-216; Nakatani et al, Bio/Technology, 1989, 7, 805-810; and Gillies et al, Bio/Technology, 1989, 7, 799-804).

An alternative mammalian expression system is that offered by the use of dihydrofolate reductase (dhfr) deficient Chinese hamster ovary (CHO) cells. The use of these cells has enabled the production of large quantities of several therapeutic proteins for research and clinical use (Kaufman et al, Mol.Cell.Biol, 1985, 5, 1750-1759; and Zettlmeissl et al, Bio/Technology, 1987, 5, 720-725). There are, however, very few instances of the use of these cells for the expression of antibodies.

WO89/01783 and Colcher *et al* Cancer Research 49:1738 (1989) describe the production of a recombinant form of B72.3, an anti-cancer antibody, in CHO cells. In both references successful production is described and the resultant antibody is shown to localise to tumours in animal models. However, until now, it was not known whether antibodies produced in CHO cells would retain binding and functionality and if they did whether this would equate with therapeutic usefulness in humans.

Antibodies are glycoproteins containing between 3 and 12% carbohydrate. The carbohydrate units are transferred to acceptor sites on the antibody chains after the heavy and light chains have combined. The major carbohydrate units are attached to amino acid residues of the constant region of the antibody. Carbohydrate is also known to attach to the antigen binding sites of some antibodies and may affect the antibody-binding characteristics by limiting access of the antigen to the antibody binding site. There are a number of roles associated with the carbohydrate units. They may affect overall solubility and the rate of catabolism of the antibody. It is also known that carbohydrate is necessary for cellular secretion of some antibody chains. It has been demonstrated that glycosylation of the constant region plays a vital role in the effector functioning of an antibody; without this glycosylation in its correct configuration, the antibody may be able to bind to the antigen but may not be able to bind for example to macrophages, helper and suppressor cells or complement, to carry out its role of blocking or lysing the cell to which it is bound.

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It has now been found that antibody glycosylated by CHO cells maintains antigen binding capability and effector functionality. This has been demonstrated in in vitro complement lysis assays and in vivo in a human patient.

The invention therefore provides an antibody having CHO glycosylation. Such antibodies may be natural, such as human antibodies, altered antibodies for example hybrid antibodies or bispecific antibodies, chimaeric or CDR-grafted antibodies.

The CHO glycosylation may be associated with the antigen binding site or other parts of the variable domain. It may alternatively or additionally be associated with the constant region. The glycosylated antibody is prepared by expression of the antibody genes in a suitably engineered CHO cell followed by recovery and if necessary, purification of the antibody from the cell culture medium.

CHO glycosylated antibodies are useful in medical therapy for treating numerous human disorders, generally as immunosuppressives more particularly for example T-cell mediated disorders including severe vasculitis, rheumatoid arthritis, systemic lupis, also autoimmune disorders such as multiple sclerosis, graft vs host disease, psoriarsis, juvenile onset diabetes, Sjogrens' disease, thyroid disease, myasthenia gravis, transplant rejection and asthma. These antibodies are also useful in treating cancer such as Non-Hodgkins lymphoma, multiple myeloma, and infectious diseases such as HIV and herpes.

The invention therefore provides the use of CHO glycosylated antibodies in the manufacture of a medicament for the treatment of any of the aforementioned disorders. Also provided is a method of treating a human being having any such a disorder comprising administering to said individual a therapeutically effective amount of a CHO glycosylated antibody.

The dosages of such antibodies will vary with the condition being treated and the recipient of the treatment, but will be in the range 1 to about 100 mg for an adult patient preferably 1 - 10 mg usually administered daily for a period between 1 and 30 days. A two part dosing regime may be preferable wherein 1 - 5 mg are administered for 5 - 10 days followed by 6 - 15 mg for a further 5 - 10 days.

Also included within the invention are formulations containing CHO glycosylated antibody. Such formulations preferably include, in addition to antibody, a physiologically acceptable diluent or carrier possibly in admixture with other agents such as other antibodies or an antibiotic. Suitable carriers include but are not limited to physiological saline, phosphate buffered saline, phosphate buffered saline glucose and buffered saline. Alternatively, the antibody may be lyophilised (freeze dried) and reconstituted for use when needed by the addition of an aqueous buffered solution as described above. Routes of administration are routinely parenteral including intravenous, intramuscular, subcutaneous and intraperitoneal injection or delivery.

A process has been developed that enables the balanced expression of the light and heavy chains of an antibody from CHO cells. Balanced expression is desirable given that the light and heavy chains are linked together in the antibody molecule in equimolar proportions. This process allows the antibody to be obtained in functional form and to be secreted in good yields. Thus the process enables sufficient quantities of functional antibody to be obtained for use in the immunotherapy of pathological disorders.

A CHO cell line as described herein is capable of producing all kinds of antibodies that generally comprise equimolar proportions of light and heavy chains. The invention therefore includes human antibodies wherein the amino acid sequences of the heavy and light chains are homologous with those sequences of antibodies produced by human lymphocytes in vivo or in vitro by hybridomas. Also included in the invention are altered antibodies such as hybrid antibodies in which

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the heavy and light chains are homologous to a natural antibody but are combined in a way that would not occur naturally. For example, a bispecific antibody has antigen binding sites specific to more than one antigen. The constant region of the antibody may relate to one or other of the antigen binding regions or may be from a further antibody. Altered antibodies, such as chimaeric antibodies have variable regions from one antibody and constant regions from another. Thus, chimaeric antibodies may be species/species chimaeras or class/class chimaeras. Such chimaeric antibodies may have one or more further modifications to improve antigen binding ability or to alter effector functioning. Another form of altered antibody is a humanised or CDR-grafted antibody including a composite antibody, wherein parts of the hypervariable regions in additon to the CDRs are transferred to the human framework. Additional amino acids in the framework or constant regions of such antibodies may be altered. Included in the definition of altered antibody are Fab fragments which are roughly equivalent to the Y branch portions of the heavy and light chains; these may be included incomplete fragments or fragments including part of the Fc region. Thus, within the scope of the invention is included, any altered antibody in which the amino acid sequence is not one which exists in nature.

A CHO cell line as described herein may be employed for the production of altered antibodies most preferably chimaeric antibodies or CDR-grafted antibodies. Particular examples of these include antibodies against T cell markers such as CD2, CD3, CD4, CD5, CD7, CD8, CD11a, CD11b, CD18, CD19, CD25, CD45 and CDw52 and especially CDR grafted antibodies against the CDw52 antigen, such as Campath-1H (Campath is a Trademark of the Wellcome Foundation Ltd) described in EP 328404 Further examples include CDR-grafted antibodies against various cancer cell marker antigens such as CD33 and CD38.

After co-transfection into recipient CHO cells, the resulting colonies may be selected using both markers. Colonies exhibiting the dual phenotype are generally capable of co-expressing both the light and heavy chains. The selectable markers may or may not be of a dominant

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nature. Examples of selectable markers for use co-transfection include adenosine deaminase (Kaufman et al, P.N.A.S., 1989, 83, 3136-40) asparagine synthetase (Cartier et al, Mol.Cell Biol., 1987, 7, 1623-28), E.coli trpB gene and Salmonella hisD gene (Hartman et al, P.N.A.S., 1988, 85, 8407-51), M2 mouse ribonucleotide reductase (Thelander et al, EMBO J, 1989, 8, 2475-79), human multidrug resistance gene (Kane et al, Gene, 1989, 84, 439-446), glutamine synthetase (Bebbington et al, DNA Cloning, Vol III, 1987, Ed. D.M. Glover, 163-188, IRL Press), xanthine guanine phosphoribosyl transferase (gpt) (Mulligan et al, Science, 1980, 209, 1422-27), hygromycin B (Santerre et al, Gene, 1984, 30, 147-156), neomycin gene (Southern et al, J. Mol. Appl.Genet., 1982, 1, 327-341), and dihydrofolate reductase (Subramani et al, Mol.Cell Biol., 1981, 1, 854-864). One particularly preferred selectable marker is dhfr which is usually employed with a parental CHO cell line of the $dhfr^-$ phenotype (Urlaub et al, P.N.A.S., 1980, 77, 4216-4220). Successfully co-transfected CHO cells will possess the $dhfr^+$ phenotype and can readily be selected by culturing the colonies on media devoid of thymidine and hypoxanthine and optionally containing methotrexate (MTX). A preferred selectable marker for use with the other of the vectors is a dominant resistance marker, such as neomycin (neo). CHO cells successfully transfected with this marker can readily be selected by culturing the colonies on media containing the antibiotic, G418, otherwise known as Geneticin.

A second system of selection and amplification is provided by the glutamine synthetase selectable marker or (GS system) which is described in WO87/04462. CHO cells which have been successfully transfected with the gene encoding the GS enzyme and the desired antibody gene can be selected by culturing colonies in media devoid of glutamine as described in PCT published application number WO87/04462.

At least one of the selectable markers preferably also provides the basis upon which the genes encoding the light and heavy chains may be amplified. In co-transfection of a CHO cell line, the vector DNAs are often integrated into the chromosome of the cell at the same locus.

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Thus, the use of only one of the selectable markers as the basis for amplification normally results in a parallel increase in the copy number of both genes. One particular selectable marker for use in this way is dhfr which enables the desired amplification to be obtained through the use of increasing concentrations of MTX. A second preferred selectable marker is GS which allows amplification by the addition of methionine sulphoximine (MSX).

The selectable markers are of course under the control of regulatory elements of DNA so as to provide for their expression. In the case of the use of dhfr as a selectable marker, the regulatory elements are preferably of a viral source, such as from DNA tumour viruses. Particularly preferred are the use of an SV40 or adenovirus major late promoter. It is particularly advantageous in this regard to remove the enhancer element from the promoter thus effectively "crippling" it. This modification allows for increased levels of gene amplification at each concentration of methotrexate selection than would otherwise occur if a strong promoter was used. In the case of the use of neo as a selectable marker, an example of a suitable promoter is the mouse metallothionein promoter.

The light and heavy chain genes may constitute genomic DNA or, preferably, cDNA, and are cloned using procedures known in the art (Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis et al, Cold Spring Harbor). The genes are also under the control of regulatory elements of DNA so as to provide for their expression. The use of the same regulatory elements for both chains is preferred so that their expression is substantially balanced. The regulatory elements may be of viral origin and examples include those mentioned above in conjunction with the expression of dhfr as a selectable marker. Another example is the use of the β-actin promoter and cognate β-actin polyadenylation signal.

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One or both of the vectors may also contain an SV40 origin of replication to allow for the vector constructs to be checked by rapid transient assay.

Construction of the expression vectors may be carried out in accordance with procedures known in the art (Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis et al, Cold Spring Harbor).

Co-transfection of the CHO cell line with the expression vectors may be carried out simply by using equimolar quantities of both vectors and standard transfection procedures, such as calcium phosphate precipitation or lipofectin. Selection of the desired co-transfected cell line may be carried out in accordance with standard procedures known for the particular selectable markers.

Culture of the CHO cell line may be carried out in serum-containing or preferably serum and protein free media. In one preferred instance where the CHO cell line is a $dhfr^+$ transformant, the medium preferably lacks hypoxanthine and/or thymidine and optionally contains MTX. Where a selectable marker is glutamine synthetase the medium preferably lacks glutamine and optionally contains MSX. Expression of both chains in substantially equimolar proportions enables optimum yields of functional antibody to be obtained. The two chains assemble within the cell and are then secreted into the culture medium as functional antibody. The resulting antibody may be purified and formulated in accordance with standard procedures.

The accompanying drawings show:

Figure 1

(a) the pLD9 construct containing expression cassettes for the 'crippled' dhfr selection/amplification marker and the Campath-1H light chain cDNA. The small box with the dashed arrow is the weakened

SV40 promoter; the larger dotted box with an arrow is the β-actin promoter; polyA refers to respectively sourced polyadenylation and termination signals; the small box with ori contains the SV40 origin of replication;

(b) the pNE316 construct containing expression cassettes for the neomycin selection marker and the Campath-1H heavy chain cDNA. The box with an arrow and MT refers to the mouse metallothionein promoter. Restriction sites indicated are:- H, HindIII; Bg, BglII; B, BamHI; R1, EcoR1.

Figure 2

Comparative determinations of the rate of Campath-1H synthesis in confluent A39 cells over 4 consecutive days. Following the [^{35}S] methionine pulse period, equal aliquots of cells (C) and culture medium (M) were immuno-precipitated and separated by SDS-PAGE. The

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position of the Campath-1H heavy and light chains are indicated (H and L arrows). There was some loss of material for the day 3 cell sample.

Figure 3

A pulse-chase experiment to determine the rate of secretion and distribution of radiolabelled Campath-1H in A39 cells. Confluent cells were pulsed with [^{35}S] methionine for 6 hours, then fresh medium containing an excess of unlabelled methionine was added. Equal aliquots of cells and culture medium were taken at the indicated time points (in hours following the end of the pulse period) and treated as described in the legend of Figure 2. The samples for the 48 and 72 hour medium time points were run on a different gel to the 6 and 24 hour points, and the tracks are only lined up relative to the position of the heavy (H) chain.

Figure 4

Shows growth of C1H 3D11* 44 in WCM5 (protein-free medium) in a 1 litre fermenter measured as cell count/ml over 90 days.

Figure 5

Shows antibody production from C1H 3D* 44 cells in WCM5 in a 1 litre fermenter measured as micrograms of antibody/ml over 80 days.

The following Examples are provided purely for illustration of the present invention.

EXAMPLE 1: Cloning of the Heavy and Light Chain cDNAs for Campath-1H

The complementarity determining regions from the rat Campath-1G monoclonal were originally grafted directly into genomic human heavy and light chain frameworks (Winter et al, Nature, 1988, 322, 323-327). These constructs were engineered for expression in the myeloma cell

line YO and resulted in yields of Campath-1H of up to 5µg/ml following 10-14 days in culture (Hale et al, Tissue Antigens, 1990, 35, 118-127 and Winter et al, Nature, 1988, 322, 323-327). The myeloma cell line TF57 (Hale et al, ibid,) was used to generate size selected cDNA fractions of 0.9-1.2kb and 1.4-1.7kb for the light and heavy chain cDNAs respectively. These were used to make EcoR1 linkered cDNA libraries in λgt10. All procedures were as described by Huynh et al (DNA Cloning, Vol I: A Practical Approach, 1984, Glover,D(Editor), IRL Press,Oxford). The libraries were screened using [^{32}P] nick translated probes specific for the variable regions to isolate full length cDNA clones. For the light chain cDNA, the 5' untranslated leader was removed up to position -32 using Bal-31 exonuclease and a HindIII linker added. For the 3' end, use was made of a unique SacI site 47bp upstream of the stop codon. A SacI-HindIII oligonucleotide pair was used to regenerate this sequence and position the HindIII site immediately after the stop codon. For the 5' end of the heavy chain cDNA, the unique NcoI site overlapping the ATG start codon was used to re-build a 29bp untranslated leader, identical to that of the light chain, using a HindIII-NcoI oligonucleotide pair. At the 3' end, the unique NaeI site 12bp downstream of the stop codon was converted into a HindIII site using linkers.

EXAMPLE 2: Construction of Vectors:

The human β-actin promoter was excised from pHβAPr-3-neo (which corresponds to pHβAPr-1-neo (Gunning et al, P.N.A.S., 1987, 84, 483-35) except that the SV40 polyadenylation/termination signal has been replaced with the respective human β-actin signals) as a 2860 bp PvuII-HindIII fragment, in which the PvuII site was subsequently converted to a BglII site using linkers. To isolate the human β-actin polyadenylation and termination signals from pHβAPr-3-neo, an SphI site 1.4kb downstream of the unique HindIII site was converted to a BamHI site using linkers. The basal dhfr vector called p104, was constructed as follows. The SphI site at position -128 in the SV40 promoter in pSV2dhfr (Subramani et al, Mol.Cell.Biol., 1981, 1,

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854-864) was converted into a SalI site to remove all enhancer elements from the promoter. The weakened dhfr expression unit was then subcloned as a SalI-BamHI fragment into the homologous sites in pSVOd (Mellon et al, Cell, 1981, 27, 279-288).

To construct pLD9, the p104 vector was digested with BamHI, phosphatased, and ligated with three other fragments consisting of the BglII-HindIII β-actin promoter, the HindIII Campath-1H light chain cDNA and the HindIII-BamHI β-actin polyA/termination signals. To construct pNH316, the construct pdBPV-MMTneo (Law et al, Mol.Cell.Biol., 1983, 3, 2110-2115) was digested with BamHI, phosphatased, and the fragment containing the neomycin gene isolated following separation on an agarose gel. This was ligated to the two β-actin fragments and the Campath-1H heavy chain cDNA. The constructs, pLD9 and pNH316 are depicted in Figure 1.

EXAMPLE 3: Expression of Campath-1H in CHO Cells:

The dhfr$^-$ CHO cell line DUK-B11 (Urlaub et al, P.N.A.S., 1980, 77, 4216-4220) was grown in Iscove's MEM supplemented with 10% fetal bovine serum, and 4μg/ml each of hypoxanthine and thymidine. 10μg of pLD9 and pNH316 was co-precipitated onto cells using the calcium phosphate method, (Gorman et al, DNA Cloning, 1985, Vol II, 143-190, Academic Press,N.Y.) and selected for the double phenotype of dhfr$^+$/neo resistance by using the medium above except that 10% dialysed serum was used, the hypoxanthine/thymidine were omitted, and G418 (Gibco) was included at 500μg/ml. In some experiments MTX was included directly in the first round selection for dhfr$^+$ transformants. Several hundred resistant colonies were pooled and assayed for the production of Campath-1H antibody in the culture medium. The average yield was 0.5μg/ml for non-amplified first round transformants.

Each pooled cell population was then cultured in the presence of 10^{-7}M MTX, and after two weeks, resistant colonies were again pooled and

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titred for Campath-1H production. There was a considerable increase in yield of up to 80-fold (Table 1). These cells were dilution cloned, screened for Campath-1H yield, and two high producer lines isolated, called A37 and 3D9 (Table 1). These were both amplified further in the presence of 10^{-6}M MTX, then dilution cloned and screened as above. The increase in expression at this second, and final, amplification stage was not so dramatic as seen previously; nevertheless, when re-fed at confluence and left for a further 4 days, the cell lines A39 and 3D11 were capable of producing up to 200µg/ml of Campath-1H.

TABLE 1

Expression Levels of Campath-1H using Stepwise Amplification

Construct	Selection stage	Accumulated Campath-1H (µg/ml)
pLD9 + pNH316	$dhfr^+$/neo basal pool	0.5
	10^{-7}M MTX amplified pool	18-40
	Cell lines A37 and 3D9	40
	10^{-6}M MTX amplified pool	60-90
	Cell line A39	100
	Cell line 3D11	150-200

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Legend to Table

Cells were allowed to reach confluence in a T-175 tissue culture flask, then re-fed with fresh 50ml of tissue culture medium and left for a further 4 days. The Campath-1H antibody that had accumulated in the medium during this period was measured by ELISA. Total cell counts on the day of assay were usually 2.5 X 10^7. The yield from the 3D11 cell line reflects a productivity of 100µg/10^6 cells/day.

The co-transfection vectors pLD9 and pNH316 were further employed to evaluate an alternative amplification strategy to the one described above. The dhfr$^-$ CHO cells were co-transfected as usual, and two days later split directly into a series of flasks containing G418 (for neomycin selection) and increasing concentrations of MTX ranging from 3 X 10^{-9}M to 10^{-7}M. Following two weeks of this selection, the number of resistant colonies were counted and pooled for each flask. When the cell populations had stabilized, they were assayed for Campath-1H antibody titres and the results are shown in Table 2. As the MTX level was increased, there was a marked decrease in the number of surviving dhfr$^+$ colonies, but they expressed proportionately more Campath-1H. Thus, in a one step direct selection at high concentrations of MTX, it is possible to isolate cell populations which produce up to 60-fold increase in antibody yield compared to cell populations selected for basal dhfr levels.

TABLE 2

Expression Levels of Campath-1H using Direct Selection

Selection (M MTX)	dhfr$^+$ colonies	Accumulated Campath-1H (µg/ml)
No MTX	500	0.5

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3×10^{-9}	40	2
10^{-8}	5	7
3×10^{-8}	5	30
10^{-7}	-	-

Legend to Table

Colonies at each MTX selection stage were pooled and assayed as described in the legend of Table 1.

This selection procedure was repeated following another co-transfection of cells, and in this instance, the entire population was selected in medium containing G418 and 3×10^{-8}M MTX. This generated a larger pool of resistant colonies which were subsequently pooled and re-amplified twice more using MTX concentrations of 6×10^{-7}M, then 3×10^{-6}M. At this stage, the cells were dilution cloned and screened for Campath-1H levels. The two highest producer cell lines isolated were capable of producing antibody levels up to 100-150µg/ml and were designated as lines 4F11 and 5E10.

The growth rates of these cell lines, and the A39/3D11 lines described above, were considerably slower than the parental non-transformed dhfr$^-$ CHO cells. This is usually a common feature of these cells once they have been engineered to express high quantities of a product gene. The yields from the 5E10 and 4F11 cell lines proved to be quite variable over time, and the latter appeared to have only a limited passage life lasting about 3 weeks before entering crisis and death. This instability was not evident at all in the other cell lines, although in general, the lines isolated from the second amplification procedure, including 5E10, were usually more fickle to culture. Of all the lines, the 3D11 coupled good growth and stability with high

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Campath-1H yields. To ensure the propagation of these features, the 3D11 cell line was dilution cloned once more to generate the 3D11* line and this similarly produced Campath-1H yields up to 200µg/ml.

EXAMPLE 4: Growth of and Production from C1H 3D11* 44 in WCM4

a) C1H 3D11* cells growing as a monolayer in Iscoves + 10% FBS Flow, non-essential amino acids, 10^{-6}M Methotrexate and antibiotics were approximately 90% confluent. These cells were removed from the plastic with trypsin/versene, washed in Iscoves medium without supplements, centrifuged and resuspended at 5 x 10^4/ml in WCM4 medium Table 3 + 0.25% peptone + 0.1% polyethylene glycol (PEG) 10,000 + 0.5% fetal boine serum (FBS) without methotrexate (MTX).

TABLE 3

Formulation for medium WCM4.

Iscoves modification of DMEM without BSA, transferrin and lecithin. Available from GIBCO Ltd., Unit 4, Cowley Mill Td. Est., Uxbridge UB8 27G. Similar to published medium (Iscoves and Melcher (1978) J. Exp. Med. 1. 47, 923) without the bovine serum albumin, pure human tranferrin, or soyabean lecithin.

+	5 ml/litre	200mM L glutamine
+	50 mg/litre	L proline
+	50 mg/litre	L threonine
+	50 mg/litre	L methionine
+	50 mg/litre	L cysteine
+	50 mg/litre	L tyrosine
+	25 mg.litre	ascorbic acid
+	0.062 mg.litre	vitamin B6
+	1.36 mg.litre	vitamin B12
+	0.2 mg/litre	lipoic acid

+	0.088 mg/litre	methyl linoleate
+	1µM	methotrexate
+	1 mg/litre	$FeSO_4$
+	1 mg/litre	$ZnSO_4$
+	0.0025 mg/litre	$CuSO_4$
+	5 mg/litre	recombinant insulin (Nucellin)
+	50,000 Iu/litre	polymyxin
+	20,000 Iu/litre	neomycin
+	0.16 mg/litre	putrescine-2 HCL.

Three 25cm^2 flasks were set up with 10ml of cell suspension + hypoxanthine (H), thymidine (T) or HT. These flasks were incubated at 36.5°C in 5% CO_2 incubator.

After six days, the flasks were pooled and added to an equal volume of WCM4 + MTX without peptone or PEG, and were transferred to a 75cm^2 flask.

These cells were used to seed a 500ml Techner spinner, incubated at 36.5°C spinning at 40 rpm. Cells continued growing serum free for a period of over five months and although it was found that the cells needed a period of adaptation, the growth rate and viability steadily improved. The population doubling time was calculated to be 73.1 hours over approximately 7 weeks; this decreased to 47.4 hours over the subsequent 20 days then stabilised. Antibody secretion remained high at levels in excess of 60 µg/ml. It was determined that the gene copy number in these cells did not decrease according to band intensity using Northern blot analysis.

In fermenters, these cells produced antibody in excess of 70µg/ml and regularly achieve levels of 100µg/ml or more. These cells are denoted C1H 3D11* 44.

b) Cells from a) above which had been growing serum-free for over 2 months were transferred to a SGi 1 litre fermenter with a stainless

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steel angled paddle turning at 70rpm. The temperature was set at 37°C, dO_2 at 10% and pH control to 7-7.2. The fermenter was seeded on day 0 with 0.22 x 10^6 cells/ml in WCM4 (Table 3) with 0.1% polyethylene glycol (PEG) 10,000 and 0.25% soy peptone, and was top gassed with O_2. The cells were routinely passaged using fresh medium and a split rate typically between 1 to 2 and 1 to 4.

On day 33 the top gassing was replaced with deep sparging which is can be expected to cause more physical damage to the cells.

On day 50 onwards WCM5 (Table 4) was used together with peptone and PEG instead of WCM4.

TABLE 4

Formulation for Medium WCM5

Iscoves modification of DMEM without BSA, transferrin or lecithin (see Table 3).

+	5 ml/litre	200mM L glutamine
+	50 mg/litre	L proline
+	50 mg/litre	L threonine
+	50 mg/litre	L methionine
+	50 mg/litre	L cysteine
+	50 mg/litre	L tyrosine
+	25 mg/litre	L ascorbic acid
+	0.062 mg.litre	Vitamin B6
+	1.36 mg.litre	Vitamin B12
+	2 mg/litre	Ferric citrate
+	1 mg/litre	Zinc sulphate
+	0.0025 mg.lit	Copper sulphate
+	50,000 IU/litre	Polymyxin
+	20,000 IU/litre	Neomycin
+	3 µl/litre	Ethanolamine

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+	0.16 mg/litre	Putrescine
+	5 mg/litre	Recombinant Insulin (Nucellin)

On day 53 the PEG was replaced with 0.1% pluronic F68. The resulting growth and antibody levels achieved are shown the the attached graphs (Figs 4 and 5), and demonstrate the capacity of the invention to allow protein-free production of antibody in excess of 100µg/ml in fermenters.

EXAMPLE 5: Analysis of the Rate of Campath-1H Synthesis and Secretion from CHO Cells:

During the course of culturing the Campath-1H producing CHO cells of Example 3, it became clear that even when they reached confluence, antibody levels continued to accumulate, with time, in the culture medium. To determine whether this was possibly a consequence of intracellular accumulation coupled to slow secretion, the rates of Campath-1H synthesis and secretion were measured using A39 cells. These analyses were performed over 3-4 consecutive days on cells which were either in growth phase, or confluent stationary phase. For the cells in either growth state, the results were identical, and data is presented only for the immuno-precipitated radiolabelled Campath-1H produced from stationary cells.

The rate of antibody synthesis was measured by pulsing the cells for a short period with [S^{35}]-methionine on each of four consecutive days, and then examining the quantity, and distribution, of immuno-precipitated material. In Figure 2, it is clear that the rate of synthesis is equally high at all time points measured. Furthermore, even by the end of this short pulse, in each case, more than half of the newly synthesized Campath-1H is already present in the medium suggesting rapid secretion. This was confirmed by the data shown in Figure 3, in which parallel cells were similarly pulsed, and the distribution of the radiolabelled Campath-1H chased over a three day period. Within 24 hours, virtually all of the cellular

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radiolabelled antibody has been chased into the medium, where it remained stable for the duration of the experiment. This demonstrates that even when the recombinant CHO cells remain stationary for long periods, the rates of Campath-1H synthesis and secretion are not diminished.

Campath-1H ELISA assay. Microtiter plates were coated with anti-human IgG and incubated with the assay sample (in culture medium). Antibody detection was visualized by using an anti-human gamma chain specific peroxidase conjugate.

Analysis of rates of Campath-1H synthesis and secretion. Cells from Example 3 were grown to confluence in 3cm tissue culture wells, then incubated for 30 minutes in methionine-free Dulbeccos's MEM containing 10% fetal calf serum. Following this, the cells were labelled in the presence of 120µCi/ml [^{35}S] methionine (>800Ci/mmol; Amersham) for the appropriate time period, then either harvested and lysed in 500µl of NP-40 lysis buffer, or incubated further in normal growth medium. Then 125µl aliquots of cell lysate or culture medium were immuno-precipitated using goat anti-human IgG (heavy chain specific; Sigma) and 10% protein-A Sepharose (Pharmacia). Samples were then separated on 10% SDS-PAGE reducing gels according to Laemmli and the signals amplified with Enhance (NEN-Dupont). The dried gels were then autoradiographed overnight.

Biological assays for functional CHO-glycosylated Campath 1H

Complement lysis assay for Campath 1H

The complement lysis assay is a measure of antibody function expressed as specific activity, determined by the ability of a CHO-glycosylated antibody of known concentration to bind to a pre-determined number of cells and effect cell lysis.

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The assay is carried out on Campath 1H from Example 4 using Karpas 422 cells (established from B-cell non-Hodgkin lymphoma cell line - Dyer et al., (1990) Blood, 75 704-714) expressing Campath antigen on the cell surface. 1.2 x 10^7 cells were loaded with radiolabel by incubating for 2 hours at 37°C in a CO_2 incubator in the presence of 600µCi of 51Cr (sodium chromate).

5.3 ml of the loaded cells in medium (total volume 23.5ml), were added to 12.5ml of normal human serum and 150µl of the mixture were pipetted into the wells of a microtitre plate.

50µl samples of the final eluate from three purification runs were mixed with the cells and incubated for 30 minutes at 4°C followed by 90 minutes at 37°C. The culture was centrifuged at 2000 rpm for 5 minutes and the radioactivity in 100µl of cell supernatant was counted on a gamma counter. Complement lysis activity in Kilo Units/ml was calculated from a standard curve of a reference preparation (1000 Units/ml).
The results are set out in Table 5.

The concentration of Campath 1H in the 50µl samples of final eluate was estimated using samples in PBS pH 7.2 read on a spectrophotometer at 280nm. The results are expressed in Table 3 as optical density in mg/ml.

From this data the specific activity: $\frac{KU/ml}{OD}$ is determined.

TABLE 5

Sample	Complement lysis Kilo Units/ml	Protein Conc mg/ml	Specific Activity
A	11.2	11.1	1.0
B	14.8	14.2	1.0
C	13.7	13.6	1.0

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The results indicate that CHO-glycosylated Campath 1H is functional.

Treatment of an individual with CHO-glycosylated Campath 1H

An individual diagnosed as having severe T-cell mediated inflammation of the joints (immobilising polyarthritis, pleuritis, abdominal pains) over five years requiring long periods of hospitalisation was treated with CHO derived Campath 1H from Example 4 using the following regime:

2mg per day over 6 days by intravenous injection
10 mg per day over subsequent 6 days by intravenous injection.

During the second 6 day treatment there was a significant symptomatic improvement. By the end of the second period the joint inflammation was much improved and a skin abscess had cleared with antibiotic treatment. Thirty days after the end of the treatment the individual was discharged.

Approximately 9 months after the initial treatment, the individual suffered a relapse with multiple joint involvement. After initial testing for sensitivity with a low dose, the individual was given a further course of treatment with 10mg/day Campath 1H for 10 days with significant improvement.

EXAMPLE 6

EXPRESSION OF HUMANISED ANTI-CD4 ANTIBODY FROM CHO CELLS

Construction of the expression vector pBan1; modification of p342-12

The complementarily determining regions from a rat IgG2b raised against human CD4 (The New England Journal of Medicine 1990 323 : 250-254) were grafted onto human heavy and light chain frameworks (Winter et al, Nature, 1988, 322 323-327).

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The cDNA encoding the humanised CD4 light chain was cloned into pLD9 [Page and Sydenham, M.A. 1991 Biotechnology 9 64-68]. The resulting plasmid was designated p2110. The humanised CD4 heavy chain was sequenced and cloned into a modified version of plasmid p342-12 [Law M-F., Byrne, J.C. and Hinley, P.M. 1983 Mol. Cell. Biol. 3 2110-2115). Plasmid p342-12 was digested with BamH1 to remove the 7.4kbp fragment containing part of the BPV-1 genome. The backbone containing the β-lactamase gene and the neomycin resistance gene under the control of the mouse metallothionine promoter was purified and religated at the BamH1 site. This plasmid was digested with HinDIII, incubated with the large fragment of DNA polymerase I to remove the HinDIII site and then religated. The β-actin expression cassette, containing the β-actin promoter immediately upstream of a unique HinDIII site followed by the polyadenylation signal, was cloned into the BamHI site of the modified p342-12 plasmid to generate pBan1.

Plasmid pBan1, therefore, consisted of the neomycin resistance gene, the β-lactamase gene and the β-actin expression cassette containing the unique HinDIII site. The cDNA encoding the humanised heavy chain was cloned into this site and the resulting plasmid containing the correctly orientated insert was designated pBanCD4H. Thus, p2110 and pBanCD4H contained a different selectable marker and co-transfection into recipient dhfr- CHO cells would permit the direct selection and isolation of $dhfr^{+}/neo^{r}$ colonies. Cells exhibiting this phenotype should express functional antiCD4 antibody and could be amplified to elevate the antibody titres.

Expression of anti-CD4 antibody in CHO cells

a) Cell culture methods.

The dhfr- CHO line DUK-B11 [Urlaub,G.and Chasin, L.A. 1980 Proc.Natl.Acad.Sci.USA77 4216-4220]was propagated in Iscoves MEM medium supplemented with 10% foetal bovine serum and 4μg each of hypoxanthine and thymidine (all Flow).After transfection,

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transformants were selected in the medium described above except that the hypoxanthine/thymidine were omitted and dialysed foetal bovine serum was used. In addition, G418 was included at 500µg/ml. To induce spontaneous amplification of sequences containing and flanking the dhfr gene, MTX was added to a concentration of 0.1µM.

b) Transfection and amplification

The dhfr- CHO cell line DUK-B11 was co-transfected with 5µg of p2110 and 5µg of pBanCD4H using the transfectam reagent under the conditions recommended by the manufacturer. Transformants were selected for the $dhfr^+/neo^r$ phenotype as described above. Several hundreds of transformants were observed and pooled. Initital titres indicated that the first round basal transformants were secreting about 0.1µg/ml/day. This pooled population was then cultured in the presence of 0.1µM MTX for about 14 days. Resistant colonies were again pooled and assayed. Expression had increased some 100 fold, the pooled, amplified colonies producing about 10-12µg/ml/day. In order to obtain stable, clonal cell lines giving high antibody titres, the resistant pools were cloned by limiting dilution in 96-well plates. Fifty single colonies were identified and assayed and the four lines giving the highest titres propagated. This process of identifying highly expressing clones within the resistant population produced a line designated D419 which expressed the anti-CD4 antibody at about 20µg/ml/day.

Characterisation of $dhfr^+/neo^r$ cell lines

i) Determination of copy number and steady state transcription levels by slot blot analysis of DNA and RNA.

Whole cell RNA and DNA was prepared from the various stages of amplification as described by Maniatis et al. [1982 Molecular

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Cloning. A Laboratory Manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York]. After fixing onto nitrocellulose filters, the nucleic acids were probed with [^{32}-P]-aATP labelled DNA sequences of the heavy chain, the dhfr gene and the β-actin gene as a control "housekeeping" gene to eliminate artifacts due to loading errors.

Inititally, the uncloned 0.1μM MTX amplified pool was compared to the first round unamplified transformants and the untransformed parental B11 cells, with the probes described. Accordingly, no DNA signal was detected in the parental line when probed with the heavy chain but a weak signal was detected for dhfr. This is due to the single, non-functional dhfr allele in the B11 cell line. As a result, no RNA signal was detected with either probe. In contrast, a strong signal was detected with both probes on RNA and DNA in the primary transformants which reflects the start of expression. A very significant increase in copy number and steady state levels of RNA of heavy chain and dhfr is observed in the uncloned amplified pool. This accurately correlates with the observed increase in expression. Steady state levels of β-actin RNA were consistent in all three lines examined.

A similar comparison was made between the four highest expressing cloned cell lines. A strong signal was detected on both the RNA and the DNA blots. However, although the DA19 line was expressing twice as much antibody as a line designated D423, this difference was not in either the copy number or steady state levels of RNA. There are two possible explanations for this observation; the first is that the DNA in the DA19 line has integrated at a site in the genome at which it is under the influence of an enhancer. However, this presumably would be reflected in elevated levels of RNA. The more likely explanation is that in the replication and duplication of the tandem arrays in the line D423, some of the copies of the dhfr/antibody cassette have undergone re-arrangement and are non-functionaland

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truncated. This is not uncommon since the site of integration of heterologous genes is often at breakpoints in the chromosomes such as telomeres which are known to be "hot spots" for such re-arrangements. This could be resolved by Northern and Southern analysis.

ii) Protein synthesis and secretion of anti-CD4 antibody in the D419 line

The clonal D419 line was labelled with ^{35}S-methionine and cysteine and the intracellular and secreted antibody extracted by immunoprecipitation with appropriate antibodies. Following electrophoresis on reducing SDS-PAGE gels, the gels were dried and the signal detected by autoradiography.

It was clear from the result that both heavy and light chain are efficiently synthesised. Intracellularly, there need not be sto chiometry between heavy and light chains since the two associate as they pass through the secretory organelles. However, close stochiometry is observed in the secreted material.

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Claims

1. An antibody for use in medical therapy wherein the antibody has CHO glycosylation associated with the antigen binding site or other parts of the variable domain and optionally has CHO glycosylation associated with the constant region.

2. An antibody according to claim 1 wherein the antibody is a human or an altered antibody.

3. An antibody according to claim 2 wherein the altered antibody is a chimaeric or a CDR-grafted antibody.

4. An antibody according to any of claims 1-3 wherein said antibody binds to a T-cell marker.

5. An antibody according to claim 4 wherein said T-cell marker is selected from CD2, CD3, CD5, CD7, CD8, CD11a, CD11b, CD18, CD19, CD25, CD45 and CD52.

6. An antibody according to claim 5 wherein said antibody binds to the T-cell marker CD4.

7. An antibody according to any of claims 1-3 wherein said antibody binds to a tumour cell marker antigen.

8. An antibody according to claim 7, wherein said cancer cell mark antigen is selected from CD33 or CD38.

9. Use of an antibody as defined in any of claims 1-6 in the manufacture of a medicament for immunosuppression.

10. Use of an antibody as defined in any of claims 1-6 for the manufacture of a medicament for the treatment of T-cell mediated disorders.

11. Use of an antibody as defined in any of claims 1-6 for the manufacture of a medicament for the treatment of autoimmune disorders.

12. Use of an antibody according to claim 11 for the treatment of severe vasculitis, rheumatoid arthritis, systemic lupus, multiple sclerosis, graft versus host disease, psoriasis, juvenile onset diabetes, Sjorgren's disease, thyroid disease, myasthenia gravis, transplant rejection or asthma.

13. Use of an antibody according to any of claims 9-12 wherein the antibody binds to the T-cell marker CD4.

14. Use of an antibody as defined in any of claims 1-3, 7 or 8 in the manufacture of a medicament for the treatment of cancer.

15. A pharmaceutical formulation comprising an antibody as defined in any of claims 1-8, in combination with a physiologically acceptable diluent or carrier.

16. A pharmaceutical formulation according to claim 15 wherein the antibody is present from 1-100 mg per dose.